Biotechnology Research Program: Strategies for Developing an Animal Model to Assess the Potential Allergenicity of Novel Proteins in the Food Supply

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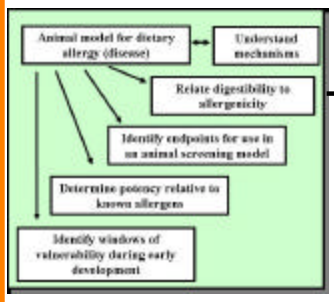
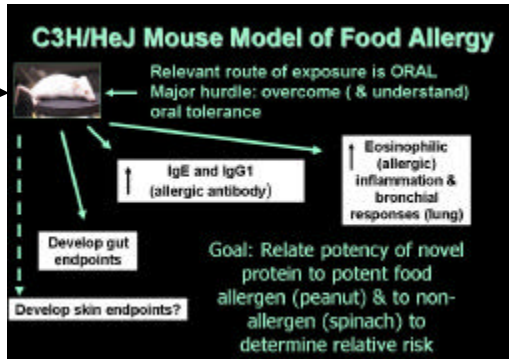
Biotechnology presents a wealth of opportunities to genetically engineer crops to improve productivity, resistance to pests and other stresses, and nutritional value. However, there is concern that novel proteins introduced into the food supply might have allergenic potential that could induce serious allergic diseases in susceptible individuals. The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) requires EPA to ensure that pesticides, including plant-incorporated protectants (PIPs), will not pose unreasonable risk of harm to human health. The incidence of food allergies in general is low (5–7% in children; 1–2% in adults), but possibly increasing. An animal model for food allergy would be a useful tool to evaluate potential allergenicity of biotechnology products and explore susceptibility issues.





Roadblock

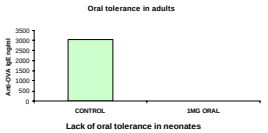
Oral Tolerance – the normal response to ingested proteins

A healthy immune system actively suppresses allergic reactions to proteins first encountered via oral exposure. To generate food allergy in mice, we are testing a variety of approaches to overcome oral tolerance, which include administering food extracts or purified food proteins to mice:

- **orally during the neonatal period (oral tolerance is deficient in very young animals)**
- **via the subcutaneous route**
- **orally in the presence of cholera toxin (an adjuvant)**
- **orally after treatment with 1-methyl-tryptophan (1-MT), a compound known to inhibit the induction of immune tolerance**
- **in enteric-coated beads to prevent digestion in the stomach**

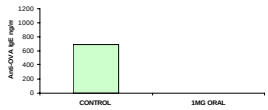
Neonatal mice lack oral tolerance

When adult mice are orally exposed to ovalbumin (an egg protein), they exhibit reduced responses to subsequent parenteral immunization (oral tolerance).

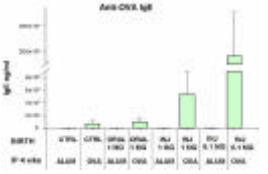


When newborn mice are orally exposed to ovalbumin, they exhibit enhanced responses to subsequent immunization (NO oral tolerance).

Probable cause of increased risk of food allergy in children.



We can sensitize newborn mice to egg protein by oral exposure or injecting into the stomach, but don't see a response unless they are immunized later by IP (intraperitoneal) injection.



Neonatal mice may help us understand the basis for susceptibility to food allergy in early life.
Neonates may not be practical for allergenicity screening because they require direct immunization and are labor/cost intensive.

Mice can differentiate allergens from non-allergens

Allergenic

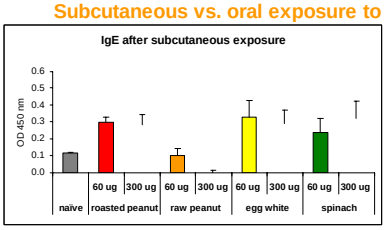
Peanut: Highly allergenic, maybe more allergenic when roasted. Proteins are stable to digestion, especially when roasted. Peanut allergy is rarely outgrown.

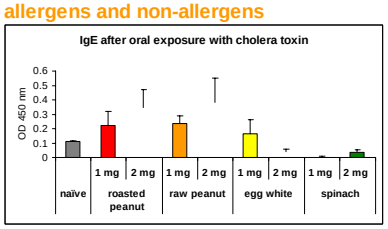
Egg white: Moderately allergenic, moderately stable to digestion. Egg allergy is frequently outgrown.

Non-allergenic

Spinach: Rarely allergenic. Highly digestible.

Subcutaneous vs. oral exposure to allergens and non-allergens





- **All food extracts elicit IgE when injected subcutaneously, whether considered allergenic or not. Subcutaneous exposure probably won't be useful in predicting allergenicity.**
- **Oral exposure to raw or roasted peanut elicits IgE responses in a dose-dependent manner.**
- **Egg white elicits very little IgE, while oral exposure to spinach elicits almost no IgE.**
- **The oral route of exposure may allow us to distinguish allergens from non-allergens, but requires adjuvant (exposure to food without cholera toxin = no IgE).**

Conclusions & next steps

Newborn mice lack oral tolerance, but do not mount sufficient responses to measure without direct immunization and are difficult to work with. May be used for identifying susceptibility factors, but probably impractical for general testing.
Subcutaneous exposure doesn't distinguish allergens from non-allergens.
Oral exposure of C3H/HeJ mouse with cholera toxin may be a good model, and appears to reflect differences in digestibility.
Digestibility issues will be addressed with encapsulated protein studies, starting soon.
Model will be applied to *Bacillus thuringiensis* toxins, which are bacterial proteins engineered into crops as pest protectants.

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